ESC European Society of Cardiology

Europace (2023) **25**, 1–9
https://doi.org/10.1093/europace/euad246

CLINICAL RESEARCH

Functional mapping to reveal slow conduction and substrate progression in atrial fibrillation

Etel Silva Garcia [1†], Ivan Lobo-Torres [1†], Juan Fernández-Armenta [1*], Diego Penela [2], Marcos Fernandez-Garcia [1], Andrea Gomez-Lopez[1], David Soto-Iglesias [2], Rafael Fernández-Rivero[1], Rafael Vazquez-Garcia [1], Juan Acosta [3], Felipe Bisbal [4,5], Lucas Cano-Calabria[1], and Antonio Berruezo [2]

[1]Department of Cardiology, Hospital Universitario Puerta del Mar, Cádiz, Spain; [2]Teknon Medical Center, Heart Institute, Barcelona, Spain; [3]Department of Cardiology, Hospital Universitario Virgen del Rocío, Sevilla, Spain; [4]Institut del Cor (iCor), Hospital Universitari Germans Trias i Pujol, Badalona, Spain; and [5]Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain

Received 11 May 2023; accepted after revision 11 August 2023; online publish-ahead-of-print 14 November 2023

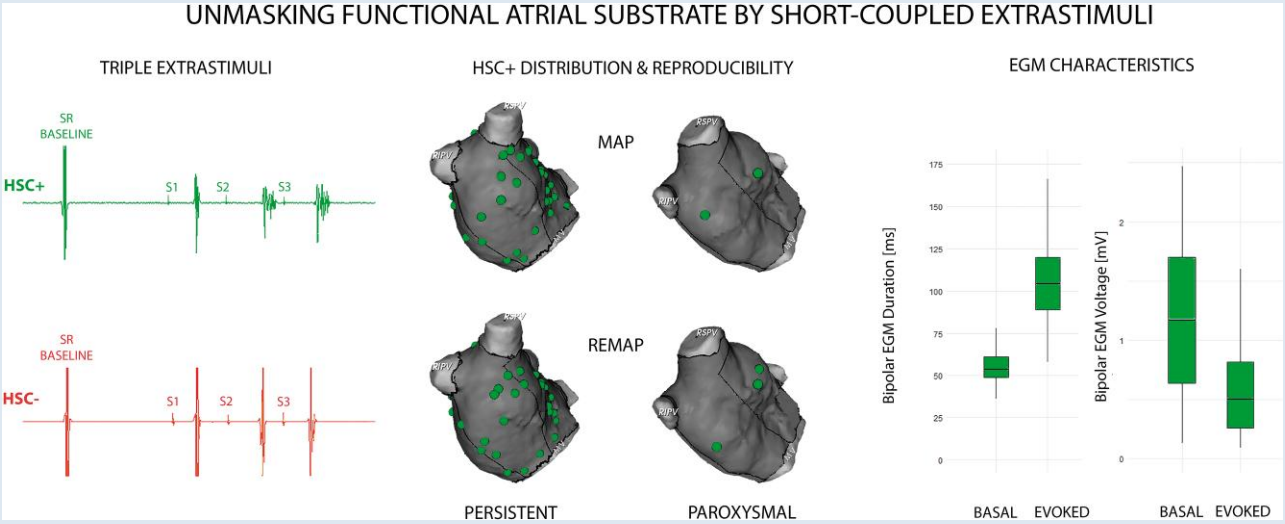
Aims The aim of our study was to analyse the response to short-coupled atrial extrastimuli to identify areas of hidden slow conduction (HSC) and their relationship with the atrial fibrillation (AF) phenotype.

Methods and results Twenty consecutive patients with paroxysmal AF and persistent AF (10:10) underwent the *first* pulmonary vein isolation procedure. Triple short-coupled extrastimuli were delivered in sinus rhythm (SR), and the evoked response was analysed: sites exhibiting double or highly fragmented electrograms (EGM) were defined as positive for HSC (HSC+). The delta of the duration of the bipolar EGM was analysed, and bipolar EGM duration maps were built. High-density maps were acquired using a multipolar catheter during AF, SR, and paced rhythm. Spatial co-localization of HSC+ and complex fractionated atrial EGMs (CFAE) during AF was evaluated. Persistent AF showed a higher number and percentage of HSC+ than paroxysmal AF (13.9% vs. 3.3%, $P < 0.001$). The delta of EGM duration was 53 ± 22 ms for HSC+ compared with 13 ± 11 (10) ms in sites with negative HSC (HSC−) ($P < 0.001$). The number and density of HSC+ were lower than CFAE during AF (19 vs. 56 per map, $P < 0.001$). The reproducibility and distribution of HSC+ in repeated maps were superior to CFAE ($P = 0.19$ vs. $P < 0.001$). Sites with negative and positive responses showed a similar bipolar voltage in the preceding sinus beat (1.65 ± 1.34 and 1.48 ± 1.47 mV, $P = 0.12$).

Conclusion Functional mapping identifies more discrete and reproducible abnormal substrates than mapping during AF. The HSC+ sites in response to triple extrastimuli are more frequent in persistent AF than in paroxysmal AF.

* Corresponding author. Tel: (+34) 956 002 358; fax: (+34) 956 266 028. *E-mail address*: juan.fernandezarmenta.sspa@juntadeandalucia.es
† Both authors equally contributed to this work.

Graphical Abstract



Keywords Atrial fibrillation • Functional mapping • Hidden slow conduction

What's new?

- Functional mapping with a short-coupled atrial extrastimuli technique could reveal the presence of slow conduction zones.
- Left atrial hidden slow conduction zones are reproducible and more extensive in persistent atrial fibrillation.

Introduction

Atrial fibrillation (AF) increases the risk of stroke, impairs quality of life, and is associated with increased overall mortality.[1,2] Pulmonary vein isolation (PVI) is the cornerstone treatment for AF, irrespective of the AF type.[2,3] A durable PVI has a pivotal role in the maintenance of sinus rhythm (SR) in paroxysmal AF, with a success rate of 80–90% at the 1-year follow-up. Nevertheless, pulmonary veins (PVs) are not the only players in the complex phenomenon of AF genesis. In fact, PVI alone achieves a moderate success rate of 40–70% in patients with persistent AF.[4]

Atrial structural and functional remodelling promotes AF progression, although the mechanisms are not yet completely understood. Several markers of atrial remodelling have been investigated as potential targets for adjuvant ablation including structural changes (areas of low voltage, regions with late gadolinium enhancement) or electrophysiological phenomena (rotational and focal activities).[5–7] The results of these new techniques have been mixed, many of which require complex technologies or extensive ablations that are not exempt from potential complications. The aim of our study was to assess the presence and extension of areas of hidden slow conduction (HSC+) unmasked by short-coupled atrial extrastimuli that may be involved in AF maintenance.

Methods

Patient population

We conducted a prospective, single-centre, experimental study including consecutive patients referred to *de novo* AF ablation between January 2022 and July 2022. All patients had documented symptomatic, drug-refractory AF and indication for ablation in accordance with ESC guidelines.[8] Persistent AF was defined in the presence of at least one AF episode that was continuously sustained beyond 7 days, including episodes terminated by cardioversion after >7 days. As part of the local institution protocol, a multidetector computed tomography (MDCT) study was obtained in all patients prior to the ablation procedure, and post-processing aimed at the reconstruction of MDCT-derived maps with left atrium wall thickness (LAWT) information was performed, as previously described.[9] Three-dimensional LAWT maps were also imported into the navigation system.[9] This study was conducted in accordance with the principles of the Declaration of Helsinki, the protocol was approved by the Local Ethics Committee, and all participants signed the written informed consent.

Electrophysiology study

High-density voltage mapping was performed using CARTO3 and a multipolar catheter (PentaRay®, Biosense Webster, Diamond Bar, CA, USA). The bandpass filter was set at 16–500 Hz, and the bipolar electrogram (EGM) was displayed at 100–200 mm/s and 0.1 mm/mV. Adequate endocardial contact was confirmed by stable EGMs and the distance to the geometry surface.

Procedural workflow is shown in *Figure 1*. Patients presenting to the lab in AF underwent first and repeat high-density maps during ongoing AF. Complex fractionated atrial EGMs (CFAE) were manually annotated in both maps to assess reproducibility. Electrical cardioversion (≤3 external biphasic shocks 200–360 J) was performed to restore SR. Functional mapping was the first step in patients presenting in SR to the lab.

Baseline and repeat high-density maps were acquired in SR with triple short-coupled extrastimuli from the left atrial (LA) appendage (filling threshold 6 mm). The triple stimuli were delivered in accordance with the atrial effective refractory period (AERP) as follows: (i) AERP + 60 ms, (ii) AERP + (40–20) ms, and (iii) AERP + (30–20) ms. Electrogram duration was manually annotated during SR and after triple extrastimuli. A colour-coded EGM duration map of the LA was constructed using the CFAE module (CARTO3) by manually annotating the earliest and latest EGM deflections. Electrograms recorded inside the PV antral area were excluded from the analysis. Reproducibility was assessed by comparing the first and repeat maps.

If AF was induced during the triple extrastimuli, a maximum of three electrical cardioversions were allowed to restore SR.

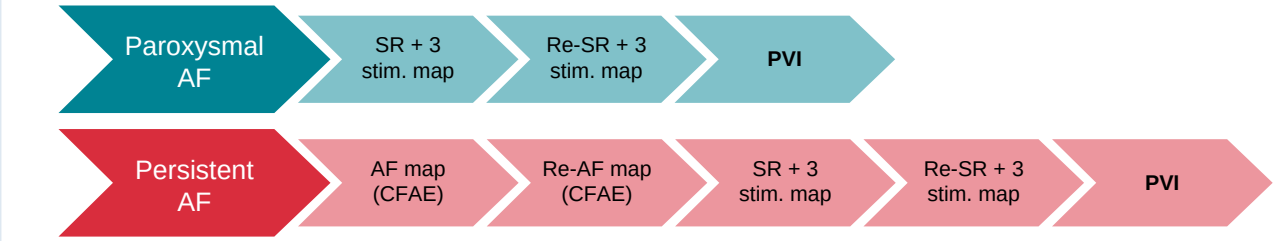


Figure 1 Procedure workflow. Consecutive electroanatomic maps during SR with triple closed-coupled extrastimuli and AF were acquired. AF, atrial fibrillation; CFAE, complex fractionated atrial electrograms; SR, sinus rhythm.

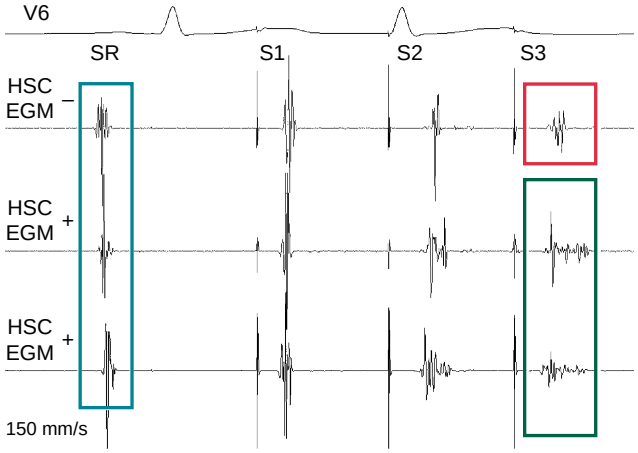


Figure 2 Schematic representation of EGMs after the application of the triple extrastimuli. EGM, electrogram; HSC, hidden slow conduction; SR, sinus rhythm.

Atrial electrogram analysis and definitions

Bipolar atrial signals during SR were divided according to their EGM waveforms:

- Normal (sharp EGMs with ≤3 positive or negative distinct peaks or EGM duration < 40 ms).[10,11]
- Complex EGMs during SR:
  - Fractionated (with >4 positive or negative distinct peaks and EGM duration ≥ 40 ms). Highly fragmented with ≥5 peaks ≥ 63 ms.[10]
  - Double potential: two or more separate deflections separated by an isoelectric interval.[10,11]
- Hidden slow conduction EGMs (HSC-EGMs) (HSC+): highly fragmented or double (showing an isoelectric line) EGMs in response to triple extrastimuli; they presented either normal or fractionated EGM in SR (*Figure 2*).

Sites were determined by visual analysis during electroanatomic map (EAM) acquisition and were analysed offline after the procedure. The delta of the duration of the bipolar EGM (third stimulated atrial EGM duration – atrial EGM duration of the sinus beat prior to triple extrastimuli) in milliseconds was recorded.

The HSC-EGM sites were represented with green dots, and highly fragmented EGMs already manifest as SR with pink dots. Electrograms with a negative response to triple extrastimuli were represented with orange dots.

During AF mapping, the multipolar catheter was sequentially positioned at various LA sites. The local EGM was considered CFAE in the case of each component having multiple deflections (positive or negative) not separated by an isoelectric line > 120 ms including continuous electric activity.[12]

Points from EAM were projected on the computed tomography reconstructions using the transformation matrix (translation and rotation) applied in the navigation system for the registration of both images. We divided the LA into six segments (*Figure 3*) using ADAS-3TM software as follows: anterior wall, septum, roof, posterior wall, left lateral wall, and PVs. Pulmonary vein EGMs were not included in the analysis.

Statistical analysis

Statistical analysis was performed using SPSS (version 23, SPSS Inc., Chicago, IL, USA). Comparisons between groups were performed using *t*-tests, and the equivalent non-parametric method and Wilcoxon rank tests will be used in cases in which the assumptions required for a *t*-test were violated. The normality of the data will be checked using box plots, normal quartile plots, and normality tests. The results will be expressed in terms of *P* values. All categorical data will be presented as frequencies and percentages, and comparisons between the randomization groups will be performed using χ^2 tests or Fisher's exact test to analyse the reproducibility of the maps. The inter-observer variability and intra-observer variability were assessed using the kappa index. Statistical significance was defined at $P < 0.05$. All data were analysed using the SPSS version 27.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Patient population

From a total of 23 patients, 20 were analysed (10 paroxysmal AF and 10 persistent AF), and three were excluded due to recurrent AF during the stimulation protocol. The mean age was 62 ± 7 years, with 75% being

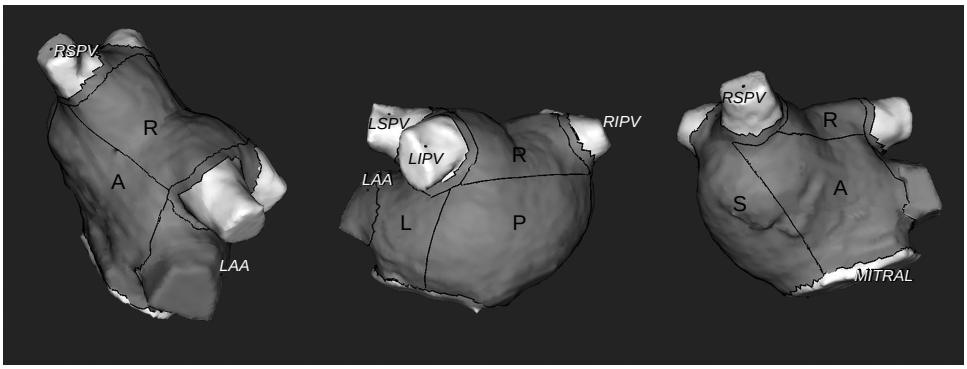


Figure 3 Left atrium segmentation. The left atrium was divided into six segments: anterior wall (A), septum (S), roof (R), posterior wall (P), left lateral wall (L), and pulmonary veins. LAA, left atrial appendage; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein.

Table 1 Baseline characteristics of the patients

	Paroxysmal AF (10)	Persistent AF (10)	*P*
Age	61 ± 7	61 ± 10	0.97
Gender (♂)	7 (70%)	8 (80%)	0.69
AHT	6 (60%)	5 (50%)	0.50
LA diameter (mm)	38 ± 9	44 ± 7	0.07
LVEF	64 ± 10	56 ± 9	0.96
AAD	9 (90%)	10 (100%)	0.50
BB	7 (70%)	9 (90%)	0.55

AAD, anti-arrhythmic drug; AF, atrial fibrillation; AHT, arterial hypertension; BB, beta-blockers; LA, left atrial; LVEF, left ventricular ejection fraction.

male. The survival rate without recurrence of AF at the 1-year follow-up was 90% for both types of AF. The baseline characteristics of the patients are detailed in *Table 1*.

Hidden slow conduction electrograms

The mean coupling interval of the first, second, and third extrastimuli was 320, 304, and 291 ms, respectively. A total of 2927 local bipolar EGMs from the first functional map were analysed: 298 (10.2%) were classified as HSC+, 2589 (88.4%) as HSC−, and 40 (1.4%) as highly fragmented or double potential EGMs in the sinus beat. The HSC+ sites were revealed after the first, second, and third extrastimuli in 16 (5.4%), 126 (42.3%), and 156 (52.3%) cases, respectively.

Overall, the mean EGM duration in SR was 58 ± 14 ms and increased to 74 ± 26 ms after triple extrastimuli ($P < 0.001$). Sites with negative and positive responses showed unremarkable differences in EGM duration in the preceding sinus beat (61 ± 15 ms vs. 56 ± 11 ms, $P < 0.001$). The HSC+ sites had a mean evoked EGM duration of 108 ± 26 ms compared with 64 ± 16 ms in the HSC− ($P < 0.001$) (*Figure 4*). The increase of EGM duration (delta) in sites with positive and negative responses was 53 ± 22 and 13 ± 11 ms, respectively ($P < 0.001$) (*Figures 4* and *5*). The baseline EGM in SR had a similar bipolar voltage in both positive and negative response areas (1.65 ± 1.34 and 1.48 ± 1.47 mV, $P = 0.12$). The bipolar voltage of the evoked potential was lower in HSC+ compared with HSC− sites (0.64 ± 0.50 and 1.11 ± 1.20 mV, respectively; $P < 0.001$) (*Figure 4* and *Table 2*).

The most frequent locations of the HSC+ were the anterior wall (89, 35.6%) and the septum (61, 16.8%).

Positive hidden slow conduction and atrial fibrillation phenotype

The HSC+ sites were more frequent in patients with persistent AF compared with paroxysmal AF (14.2% vs. 3.2%, $P < 0.001$), with a mean of 24 ± 19 and 3 ± 4 and HSC+ sites per map, respectively ($P < 0.001$) (*Figure 6*). Electrogram characteristics at baseline and after triple extrastimuli are detailed in *Table 3*. There were no differences in the location of HSC+ sites according to the AF phenotype ($P = 0.10$).

Reproducibility of functional mapping

No significant differences were observed in the number (mean of 15 ± 18 and 14 ± 17, respectively; $P = 0.44$) and distribution ($P = 0.19$) of HSC+ sites between the first and repeat maps. (*Figure 7*) Like the first map, the most frequent location of HSC+ sites in the remap was the anterior wall (41.5%). After analysing the variability on the interpretation of the repeat maps using the kappa index, we found a value of 0.96 for intra-observer variability and 0.95 for inter-observer variability, demonstrating a strong level of agreement among observers in both contexts.

Functional and complex fractionated atrial electrogram map correlation

There were significantly higher amounts of CFAE (619, mean of 62 ± 21 CFAE per map) than HSC+ (265, mean of 24 ± 19 per map; $P = 0.002$). Despite similar amounts of recorded CFAE between first and repeat maps ($P = 0.47$), there were remarkable differences in distribution ($P < 0.001$). The most frequent locations of CFAE in the first map were the anterior wall (27%), roof (26.5%), and posterior wall (22.9%), which was not consistent in the repeat map ($P < 0.001$), showing a higher presence of CFAE at the anterior wall (32.4%), roof (24.7%), and septum (20.4%) (*Figure 7*).

The distribution of CFAE and HSC+ was not consistent ($P < 0.001$), as the most frequent location of HSC+ in patients with persistent AF was the anterior wall to a higher extent (38.9%), followed by the septum (22.1%) and the posterior wall (15.4%) ($P < 0.001$).

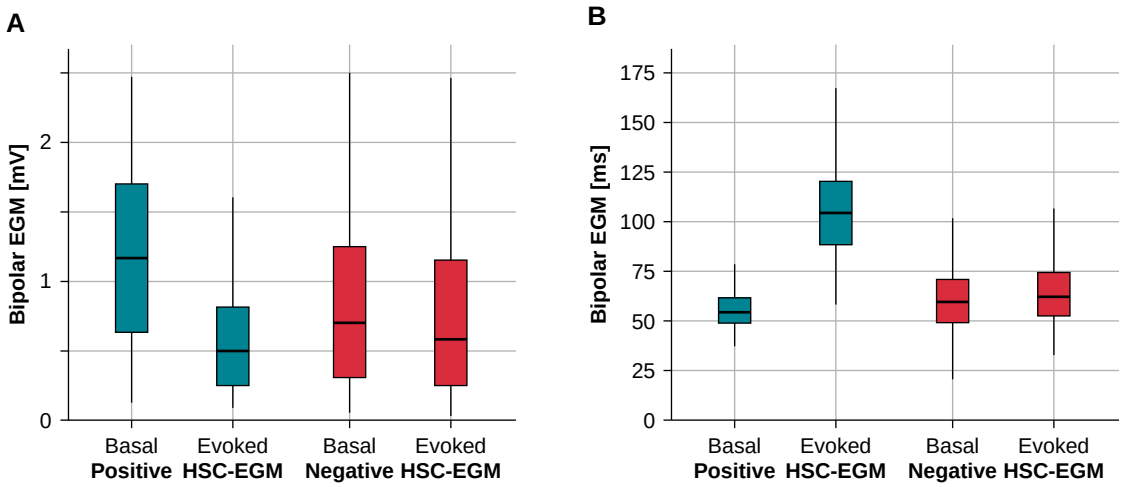


Figure 4 Electrogram characteristics of sites with positive (turquoise boxes) and negative (red boxes) responses to triple atrial extrastimuli. Bipolar voltage (*A*) and EGM duration (*B*) of the preceding SR and after the third extrastimulus. The middle horizontal line represents the median, the box represents the first and third interquartile range, and the vertical line represents the non-outlier range. EGM, electrogram; HSC, hidden slow conduction; SR, sinus rhythm.

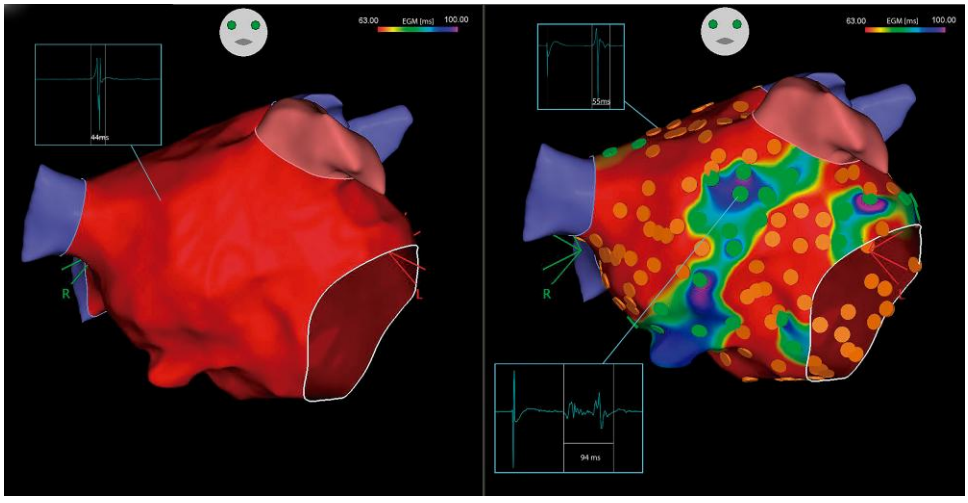


Figure 5 Electrogram duration maps. The left panel shows the colour-coded EGM duration map during SR. The right panel shows the evoked response colour-coded EGM duration map. An area of abnormal prolonged EGMs is observed in the anterior region of the left atrium in response to triple atrial extrastimuli. EGM, electrogram; SR, sinus rhythm.

Discussion

Main findings

The main findings of the study can be summarized as follows: (i) short-coupled atrial extrastimuli unmasked highly fragmented or double atrial evoked EGMs in patients with AF; (ii) HSC+ detection was reproducible and was more frequently observed in persistent AF; (iii) HSC+ sites were smaller and did not consistently co-localize with areas of complex EGMs recorded during AF; and (iv) sites with negative and positive responses showed similar EGM characteristics in the preceding sinus beat.

Electrogram fractionation and atrial fibrillation progression

Fibrosis is the main determinant of EGM fractionation.[13] Electrical remodelling can precede fibrosis, characterized by the reduction in connexin expression that results in reduced intercellular coupling with electrical impulse conduction impairment.[14] Areas with fibrosis have lower bipolar voltage, lower conduction velocity, and a higher degree of EGM fractionation. Areas of interstitial fibrosis are more prevalent in patients with AF, are age related, and represent a potential arrhythmia substrate.[15–17] In line with previous studies, we observed higher

Table 2 Global characteristics at baseline and after evoked response

	Baseline		After evoked response		
	Voltage	Duration	Voltage	Duration	Delta
Positive	1.65 ± 1.33	56 ± 11*	0.64 ± 0.5*	108 ± 26*	53 ± 22*
Negative	1.48 ± 1.47	60 ± 15	1.11 ± 1.20	64 ± 16	13 ± 11

*$P < 0.001$, positive vs. negative.

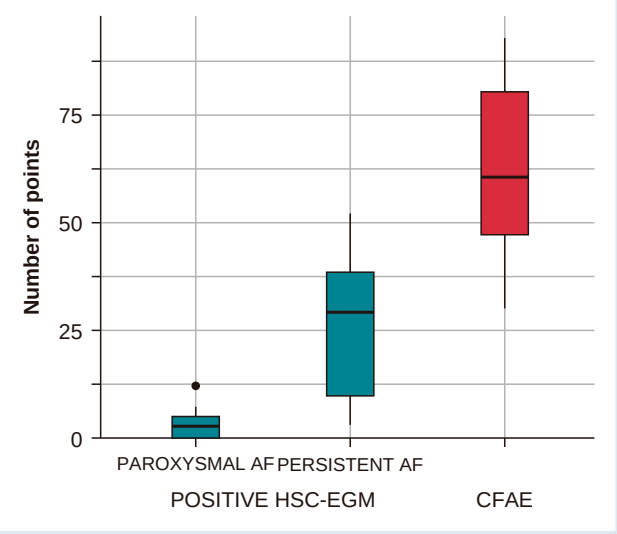


Figure 6 Box plots (turquoise boxes) of sites with HSC-EGMs in patients with paroxysmal and persistent AF. The red box plot represents the number of CFAE in patients with persistent AF. The middle horizontal line represents the median, the box represents the first and third interquartile range, and the vertical line represents the non-outlier range. AF, atrial fibrillation; CFAE, complex fractionated atrial electrograms; HSC-EGM, hidden slow conduction electrogram.

functional conduction slowing in response to closed-coupled extrastimuli in patients with persistent AF (14% vs. 3% in paroxysmal AF), suggesting advanced atrial remodelling. Although the SR bipolar voltage of the positive and negative response sites was similar, the evoked response of the HSC sites showed significantly lower bipolar voltage; HSC+ may unmask conduction abnormalities and represent an early marker of tissue fibrosis, otherwise unidentifiable during mapping in SR. Remarkably, sites with positive and negative responses showed a similar bipolar voltage and duration in SR, therefore not distinguishable without functional mapping.

Electrogram fractionation during sinus rhythm and atrial fibrillation

Electrogram fractionation during AF depends on structural and functional factors that are not always associated with the arrhythmia maintenance mechanism and also usually shows dynamic changes depending on the AF cycle length.[18,19] As expected, in line with previous works, we observed that sites with fractionation during AF changed between the two consecutive AF maps. Conversely, HSC-EGMs were consistent in terms of quantity and distribution.

Considering that CFAE during AF might be functional or occasionally bystanders that do not promote AF maintenance, some authors have investigated fractionation during sinus or stimulated rhythm and AF. Jadidi *et al.*[20] described the locations of areas of continuously fractionated atrial EGMs during AF and then correlated these areas with the locations of fractionated atrial potentials during SR and coronary sinus (CS) pacing in the same patients. There was little or no correlation between the locations of continuously fractionated EGMs during AF, during SR, and/or during CS pacing. Fractionation in SR and CS pacing was mostly caused by wave collision.[20] Similar results were obtained for others.[21] Recently, Huang *et al.*[22] related prolonged fractionation areas with low-voltage areas, with discordance in prolonged fractionation areas on the SR vs. AF map in patients with no/little underlying low-voltage substrates during SR.

There are marked differences between these works and this study. First, the definition for fragmented EGMs used in these previous studies was ≥4–5 deflections or EGMs with a delayed low-voltage component following a first component without considering EGM duration. We have focused on highly fragmented EGMs with ≥5 deflections and duration ≥ 63 ms. More recently, a study by Jadidi *et al.*[23] observed that in most of the termination sites during non-paroxysmal AF ablation, fragmented EGMs with delayed components were present in SR. Using a more restricted definition of fragmented EGMs in which voltage and duration (≥50 ms) are considered, it has been observed that they could be a potential target for AF ablation.[24] The results of targeting these complex EGMs were similar to the stepwise approach with lower requirements.[25]

Functional atrial mapping

Functional mapping could be useful to unmask EGMs suggestive of slow conduction, otherwise not evident during SR. Recently, Wong *et al.*[26] observed a higher proportion of complex signals at 300 ms pacing compared with 600 ms pacing. It is well known that the coupling interval of the stimulus is crucial in the formation of complex EGMs, allowing us to unmask dispersion in the propagation velocity between neighbouring fibres.[27]

Frontera *et al.*[28] recently analysed slow conduction corridors, defined as regions with conduction velocity < 50 cm/s, more frequent in patients with persistent AF, suggesting re-entry zones that generate substrates for the maintenance of AF. However, a relationship between these slow conduction corridors and the fractional and low-voltage EGMs has not been established. In a previous study, they established slow conduction as the main mechanism for fractioning EGMs with an amplitude of >0.5 mV (areas not usually considered diseased tissue).[15]

Short-coupled stimulation is used for slow conduction identification during ventricular tachycardia (VT) substrate mapping.[29] Abnormal ventricular areas related to VT display decremental conduction. The double ventricular extrastimuli technique permits the identification of HSC, improving arrhythmia substrate identification.[30] Unlike what happens during AF, in which the fractionation sites can be pivot points of

Table 3 Electrogram characteristics of paroxysmal and persistent

		Duration (ms)		
		Baseline	**Evoked response**	**Delta**
Paroxysmal	Positive	65 ± 16**	127 ± 33*,**	63 ± 27*,**
	Negative	65 ± 15	65 ± 14	11 ± 9**
	Highly fragmented	101 ± 29		
Persistent	Positive	55 ± 10	106 ± 24*	51 ± 21*
	Negative	56 ± 15	64 ± 17	14 ± 12
	Highly fragmented	83 ± 15		
	Double potential	70 ± 49		

*$P < 0.001$, positive vs. negative.
**$P < 0.001$, positive paroxysmal vs. positive persistent.

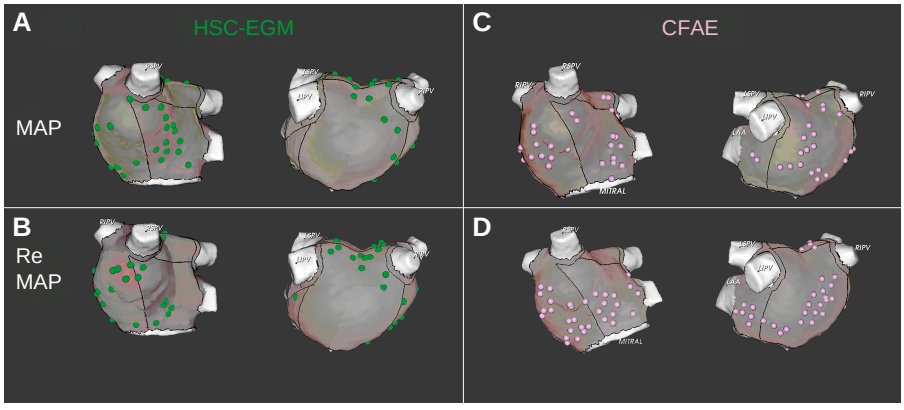


Figure 7 Hidden slow conduction electrogram reproducibility. This image illustrates the reproducibility of EGM fractionation during functional mapping or AF. Electrograms showing a positive response to short-coupled extrastimuli (HSC-EGMs) in two consecutive maps are shown in (*A*) and (*B*) (green dots). (*C*) and (*D*) show sites with CFAE (pink dots) in two consecutive maps during AF from the same patient. AF, atrial fibrillation; CFAE, complex fractionated atrial electrograms; EGM, electrogram; HSC, hidden slow conduction; LAA, left atrial appendage; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein.

wavelets or sites of wave collision, the appearance of fractionation when stressing intra-atrial conduction with short coupling mainly expresses areas of slow or poor conduction cell coupling. Analysis of the response to closely coupled atrial extrastimuli could identify areas of slow conduction that can promote re-entry and AF maintenance.

Complex atrial electrogram distribution and reproducibility

Saghy *et al.*[21] observed that there was no positive correlation between CFAE and fractionated EGMs in SR. Most of the fractionated EGMs in SR were located in the anterior wall and septal segment. Huang *et al.*[22] found that the segments with the greatest fractionation were the anterior wall, followed by the posterior wall and the septum. Frontera *et al.*[28] located the slow conduction channels (measured by conduction velocity) preferentially in the anterior wall. Recent data suggest that the proximity of the aortic root may be related to the substrate for left anterior wall atrial arrhythmias.[31] Although the detection of HSC-EGMs by triple extrastimuli has not been previously studied, in line with previous works, the most frequent location of the HSC-EGM was the anterior wall, followed by the LA septum. No differences were found in the spatial distribution of the HSC+ sites according to the AF phenotype, but they cannot be ruled out considering the small sample size.

The triple extrastimuli technique to unmask HSC-EGMs seems robust and reproducible. It shows consistency in the initial map and in the remap for the characteristics, number, and spatial distribution of HSC-EGMs.

Clinical implications

Novel ablation targets beyond PVI may be required to achieve better outcomes in the persistent AF population. Pre-defined linear lesion sets and ablation of CFAE have been proposed but have failed to prove benefits in terms of AF recurrence.[32] Recently, the multicentre ERASE-AF trial showed that targeting low-voltage areas reduces recurrences at 12 months.[33] However, only 35% of the patient population showed low-voltage areas. In this series, all patients with persistent AF showed HSC-EGMs. Only 7.7% of sites with positive responses were located in areas of low voltage (<0.5 mV) during SR. The bipolar voltage of the evoked potential was lower in HSC sites (0.64 ± 0.50 mV vs. 1.11 ± 1.20 mV). Functional mapping with the triple extrastimuli

technique could allow the early identification of the arrhythmogenic substrate, a potential target for ablation.

Study limitations

The study includes a relatively small sample size. Triple extrastimuli were delivered from the LA appendage aiming to generate a non-physiological activation wavefront to maximize the sensitivity to identify areas of functional slow conduction. Future comparisons of the evoked response with different pacing sites are needed. In the present study, we do not obtain magnetic resonance imaging to compare the distribution of HSC+ with hyperenhancement areas.

Conclusions

Highly fragmented or double atrial EGMs recorded in response to closed-coupled triple extrastimuli are reproducible and more common in persistent AF. Regions with positive evoked responses were less extensive and had limited overlap with the regions of CFAE. This type of evoked response (HSC+) could reveal the presence of slow conduction zones and/or interstitial fibrosis and could be considered a potential target for ablation of persistent AF. Further studies are needed to confirm the results of this work, which should be interpreted as hypothesis-generating. A randomized trial is currently in progress to assess the potential benefits of targeting these areas for ablation.

Funding

This study has been funded in part by the Health Council of the Andalusian Regional Government through the project 'PI-0057–2017' and co-funded by the European Regional Development Fund/European Social Fund. Adas3D Medical SL has supported this text as a funding source for the first author E.S.G.

Conflict of interest: J.F.-A.: Consultant for Biosense Webster. F.B.: Consultant for Biosense Webster, Abbott, and Biotronik. D.S.-I.: Employee of Biosense Webster. All remaining authors have declared no conflicts of interest.

Data availability

The data that support the findings of this study are available from the corresponding author, J.F.-A., upon reasonable request.

References

1. Odutayo A, Wong CX, Hsiao AJ, Hopewell S, Altman DG, Emdin CA. Atrial fibrillation and risks of cardiovascular disease, renal disease, and death: systematic review and meta-analysis. *BMJ* 2016;**354**:i4482.
2. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomstrom-Lundqvist C *et al.* 2020 ESC guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J* 2021;**42**:373–498.
3. Haissaguerre M, Jais P, Shah DC, Takahashi A, Hocini M, Quiniou G *et al.* Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N Engl J Med* 1998;**339**:659–66.
4. Clarnette JA, Brooks AG, Mahajan R, Elliott AD, Twomey DJ, Pathak RK *et al.* Outcomes of persistent and long-standing persistent atrial fibrillation ablation: a systematic review and meta-analysis. *Europace* 2018;**20**:f366–76.
5. Mohanty S, Mohanty P, Di Biase L, Trivedi C, Morris EH, Gianni C *et al.* Long-term follow-up of patients with paroxysmal atrial fibrillation and severe left atrial scarring: comparison between pulmonary vein antrum isolation only or pulmonary vein isolation combined with either scar homogenization or trigger ablation. *Europace* 2017;**19**:1790–7.
6. Bisbal F, Benito E, Teis A, Alarcon F, Sarrias A, Caixal G *et al.* Magnetic resonance imaging-guided fibrosis ablation for the treatment of atrial fibrillation: the ALICIA trial. *Circ Arrhythm Electrophysiol* 2020;**13**:e008707.
7. Tilz RR, Lenz C, Sommer P, Roza MS, Sarver AE, Williams CG *et al.* Focal impulse and rotor modulation ablation vs. pulmonary vein isolation for the treatment of paroxysmal atrial fibrillation: results from the FIRMAP AF study. *Europace* 2021;**23**:722–30.
8. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomstrom-Lundqvist C *et al.* 2020 ESC guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): the Task Force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. *Eur Heart J* 2021;**42**:373–498.
9. Teres C, Soto-Iglesias D, Penela D, Jauregui B, Ordonez A, Chauca A *et al.* Personalized paroxysmal atrial fibrillation ablation by tailoring ablation index to the left atrial wall thickness: the 'Ablate by-LAW' single-centre study-a pilot study. *Europace* 2022;**24**:390–9.
10. Frontera A, Takigawa M, Martin R, Thompson N, Cheniti G, Massoullie G *et al.* Electrogram signature of specific activation patterns: analysis of atrial tachycardias at high-density endocardial mapping. *Heart Rhythm* 2018;**15**:28–37.
11. Lellouche N, Buch E, Celigoj A, Siegerman C, Cesario D, De Diego C *et al.* Functional characterization of atrial electrograms in sinus rhythm delineates sites of parasympathetic innervation in patients with paroxysmal atrial fibrillation. *J Am Coll Cardiol* 2007;**50**:1324–31.
12. Ciaccio EJ, Biviano AB, Whang W, Vest JA, Gambhir A, Einstein AJ *et al.* Differences in repeating patterns of complex fractionated left atrial electrograms in longstanding persistent atrial fibrillation as compared with paroxysmal atrial fibrillation. *Circ Arrhythm Electrophysiol* 2011;**4**:470–7.
13. Jacquemet V, Henriquez CS. Genesis of complex fractionated atrial electrograms in zones of slow conduction: a computer model of microfibrosis. *Heart Rhythm* 2009;**6**:803–10.
14. Jansen HJ, Bohne LJ, Gillis AM, Rose RA. Atrial remodeling and atrial fibrillation in acquired forms of cardiovascular disease. *Heart Rhythm O2* 2020;**1**:147–59.
15. Frontera A, Limite LR, Pagani S, Cireddu M, Vlachos K, Martin C *et al.* Electrogram fractionation during sinus rhythm occurs in normal voltage atrial tissue in patients with atrial fibrillation. *Pacing Clin Electrophysiol* 2022;**45**:219–28.
16. Marrouche NF, Wilber D, Hindricks G, Jais P, Akoum N, Marchlinski F *et al.* Association of atrial tissue fibrosis identified by delayed enhancement MRI and atrial fibrillation catheter ablation: the DECAAF study. *JAMA* 2014;**311**:498–506.
17. Bisbal F, Baranchuk A, Braunwald E, de Luna A B, Bayes-Genis A. Atrial failure as a clinical entity: JACC review topic of the week. *J Am Coll Cardiol* 2020;**75**:222–32.
18. Atienza F, Calvo D, Almendral J, Zlochiver S, Grzeda KR, Martínez-Alzamora N *et al.* Mechanisms of fractionated electrograms formation in the posterior left atrium during paroxysmal atrial fibrillation in humans. *J Am Coll Cardiol* 2011;**57**:1081–92.
19. Rostock T, Rotter M, Sanders P, Takahashi Y, Jaïs P, Hocini M *et al.* High-density activation mapping of fractionated electrograms in the atria of patients with paroxysmal atrial fibrillation. *Heart Rhythm* 2006;**3**:27–34.
20. Jadidi AS, Duncan E, Miyazaki S, Lellouche N, Shah AJ, Forclaz A *et al.* Functional nature of electrogram fractionation demonstrated by left atrial high-density mapping. *Circn Arrhythm Electrophysiol* 2012;**5**:32–42.
21. Saghy L, Callans DJ, Garcia F, Lin D, Marchlinski FE, Riley M *et al.* Is there a relationship between complex fractionated atrial electrograms recorded during atrial fibrillation and sinus rhythm fractionation? *Heart Rhythm* 2012;**9**:181–8.
22. Huang T, Chen J, Muller-Edenborn B, Mayer L, Eichenlaub M, Moreno Weidmann Z *et al.* Validating left atrial fractionation and low-voltage substrate during atrial fibrillation and sinus rhythm-A high-density mapping study in persistent atrial fibrillation. *Front Cardiovasc Med* 2022;**9**:1000027.
23. Jadidi A, Nothstein M, Chen J, Lehrmann H, Dossel O, Allgeier J *et al.* Specific electrogram characteristics identify the extra-pulmonary vein arrhythmogenic sources of persistent atrial fibrillation—characterization of the arrhythmogenic electrogram patterns during atrial fibrillation and sinus rhythm. *Sci Rep* 2020;**10**:9147.
24. Yang G, Yang B, Wei Y, Zhang F, Ju W, Chen H *et al.* Catheter ablation of nonparoxysmal atrial fibrillation using electrophysiologically guided substrate modification during sinus rhythm after pulmonary vein isolation. *Circ Arrhythm Electrophysiol* 2016;**9**:e003382.
25. Yang B, Jiang C, Lin Y, Yang G, Chu H, Cai H *et al.* STABLE-SR (electrophysiological substrate ablation in the left atrium during sinus rhythm) for the treatment of nonparoxysmal atrial fibrillation: a prospective, multicenter randomized clinical trial. *Circ Arrhythm Electrophysiol* 2017;**10**:e005405.
26. Wong GR, Nalliah CJ, Lee G, Voskoboinik A, Prabhu S, Parameswaran R *et al.* Dynamic atrial substrate during high-density mapping of paroxysmal and persistent AF: implications for substrate ablation. *JACC Clin Electrophysiol* 2019;**5**:1265–77.
27. Saumarez RC, Slade AKB, Grace AA, Sadoul N, Camm AJ, McKenna WJ. The significance of paced electrogram fractionation in hypertrophic cardiomyopathy. *Circulation* 1995;**91**:2762–8.
28. Frontera A, Pagani S, Limite LR, Peirone A, Fioravanti F, Enache B *et al.* Slow conduction corridors and pivot sites characterize the electrical remodeling in atrial fibrillation. *JACC Clin Electrophysiol* 2022;**8**:561–77.
29. Jais P, Maury P, Khairy P, Sacher F, Nault I, Komatsu Y *et al.* Elimination of local abnormal ventricular activities: a new end point for substrate modification in patients with scar-related ventricular tachycardia. *Circulation* 2012;**125**:2184–96.
30. Acosta J, Andreu D, Penela D, Cabrera M, Carlosena A, Korshunov V *et al.* Elucidation of hidden slow conduction by double ventricular extrastimuli: a method for further

arrhythmic substrate identification in ventricular tachycardia ablation procedures. *Europace* 2018;**20**:337–46.
31. Alderete J, Penela D, Soto-Iglesias D, Marti-Almor J, Falasconi G, Teres C *et al.* The critical isthmus of left atrial anterior wall flutter is mostly circumscribed into a small area immediately behind the aortic root. Implications for catheter ablation. *Europace* 2023; **25**:euad122.718.
32. Verma A, Jiang CY, Betts TR, Chen J, Deisenhofer I, Mantovan R *et al.* Approaches to catheter ablation for persistent atrial fibrillation. *N Engl J Med* 2015; **372**:1812–22.
33. Huo Y, Gaspar T, Schönbauer R, Wójcik M, Fiedler L, Roithinger FX *et al.* Low-voltage myocardium-guided ablation trial of persistent atrial fibrillation. *NEJM Evidence* 2022;**1**: EVIDoa2200141.